RESEARCH ARTICLE

Complete mitochondrial genomes from two species of Chinese freshwater crabs of the genus *Sinopotamon* recovered using next-generation sequencing reveal a novel gene order (Brachyura, Potamidae)

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Abstract

Recent morphological and molecular evidence has challenged classical interpretations of eubrachyuran phylogeny and evolution. Complete mitochondrial genomes of two species of potamid freshwater crabs, *Sinopotamon yaanense* and *Sinopotamon yangtsekiense* were obtained using next-generation sequencing. The results revealed a novel gene order with translocations of a five-gene block and a tRNA gene in comparison to available brachyuran mitochondrial genomes. DNA sequence comparisons position the Potamidae, a primary freshwater crab family, outside of the clade for the traditional heterotreme families, and closer to the clade that includes the thoracotreme families of grapsoid and ocypodoid crabs. Mitogenomic comparisons using rapid next-generation sequencing and a much wider taxonomic sample are required for a high-resolution examination of the phylogenetic relationships within the Eubrachyura.

Keywords

Eubrachyuran, gene rearrangement, mitochondrial genome, primary freshwater crab, *Sinopotamon*

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Introduction

Brachyuran crabs are one of the most species-rich and economically important groups in extant crustaceans with about 7200 species described (Castro et al. 2015), of which two major groups, Heterotremata and Thoracotremata, are collectively referred to as the Eubrachyura (Guinot 1977; de Saint-Laurent 1980; Ahyong et al. 2007; Tsang et al. 2014). However, interrelationships of eubrachyurans still remain controversial when considering published nuclear DNA and/or mitochondrial DNA (mtDNA) and morphological analyses, especially regarding relationships among families of primary freshwater crabs and between them and other eubrachyurans (Schubart et al. 2000; Von Sternberg and Cumberlidge 2001; Cumberlidge et al. 2008; Ji et al. 2014; Tsang et al. 2014; Xing et al. 2016). Inadequate taxon sampling and phylogenetic data may mislead tree reconstruction. Mitochondrial genome (mitogenome) contains rich signals from both sequence and arrangement of 13 protein-coding genes (PCG), two rRNA genes, 22 tRNA genes and an AT-rich region (main non-coding region, mNCR) in a closed circular DNA molecule (Boore et al. 1998; Boore 1999; Sun et al. 2003). Therefore, they are considered powerful markers for resolving ancient phylogenetic relationships (Boore 1999). Nevertheless, the number of complete mitogenomes of brachyurans is limited to 52 species published to date, which is not commensurate with the extant species diversity for the Brachyura (including several species with incomplete mitogenomes; Yamauchi et al. 2003; Segawa and Aotsuka 2005; Sun et al. 2005; Ji et al. 2014; Shi et al. 2015; Wang et al. 2016; Xing et al. 2016). Meanwhile, knowledge of mitogenomes from primary freshwater crabs remains scant, and only three species have been sampled (including an incomplete sequence; Segawa and Aotsuka 2005; Ji et al. 2014; Wang et al. 2016).

Next-generation sequencing (NGS), combined with bioinformatic annotation, is becoming increasingly common for recovering animal mitogenome sequences and allows a rapid amplification-free sequencing (Jex et al. 2010). It has been used for recovering entire mitogenomes of decapod crustaceans, including brachyurans (Gan et al. 2014a, b, c, d, e; Tan et al. 2015; Xing et al. 2016). In the present study, two new mitogenomes of *Sinopotamon* species, *S. yaanense* (Chung and Ts'ao, 1962) and *S. yangtsekiense* Bott, 1967 from the family Potamidae are reported using NGS through two different strategies, transcriptome and total genomic DNA, respectively. The gene arrangements for the two mitogenomes were then compared with those of available brachyuran mitogenomes.

Sinopotamon yaanense and *S. yangtsekiense*, as two representatives of the endemic genus *Sinopotamon* occur in China, are distributed in the middle and lower reaches of the Yangtze River Basin, respectively (Dai 1999; Fang et al. 2013). When refer to the phylogenetic relationships among *Sinopotamon* species (Ji et al. 2016), the former belongs to a distinct clade ranged in Sichuan Basin and its surrounding mountains, with rounded lobes of the last segment of the male first gonopods (G1) (Dai 1999; Ji et al. 2016). While the latter species have acute lobes of the last segment of G1, is clustered together with those occurring in middle reaches of the Yellow River and the upper and

middle reaches of the Huaihe River (Fang et al. 2013, 2015; Ji et al. 2016). Along with the relationships reconstructed by morphological data and DNA barcoding involving broader taxon sampling (Zhou et al. unpublished), the monophyly of *Sinopotamon* crabs is therefore challenged (Ji et al. 2016; Shih et al. 2016). In this study, we provide a thorough description of mitogenomes of these two *Sinopotamon* species, and present independent molecular evidence for phylogenetic relationships among related groups.

Materials and methods

Sample collection

Specimens of *S. yaanense* and *S. yangtsekiense* were collected by hand from mountain streams of the Emei Mountain in Sichuan Province, China (29°36'3"N; 103°19'59"E) and Ningguo, Anhui Province, China (30°38'21"N; 118°59'27"E), respectively. These specimens were preserved in 95% ethanol and identified utilizing morphological information presented in Dai (1999) under a stereo dissection microscope (Nikon SMZ645). Voucher specimens of *S. yaanense* (catalogue number EMMZ06) and *S. yangtsekiense* (catalogue number CJ01) were deposited in the Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University (NNU), Nanjing, China.

RNA and DNA extraction, genome sequencing, and PCR

Next-generation transcriptome sequencing and next-generation total genomic DNA sequencing were used to obtain mitogenomes of the two *Sinopotamon* species. For transcriptome sequencing, total RNA of *S. yangtsekiense* was extracted from fresh tissue of one individual using the TRIzol (Takara). After determining the RNA quality, the sample was enriched by Oligo (dT) and broken into short RNA fragments. The cDNA library was then prepared and sequenced on the Illumina Hi-Seq 2000 platform (BGI). All raw data were processed to remove adaptors and clean data were assembled de novo using Trinity (Grabherr et al. 2011). Finally, unigene sequences resulting from the assemblies were identified by BLAST alignment against the nucleotide database at the National Center for Biotechnology Information (NCBI) or by comparison with other published brachyuran sequences.

For total genomic DNA sequencing, total genomic DNA of *S. yaanense* was extracted using Cell and Tissue DNA Extraction Kit (Generay Biotech). The samples were sequenced on the Illumina HiSeq 4000 platform (BGI). The sequencing libraries with average insert sizes of approximately 300 bp were prepared, and then sequenced as 150 bp paired-end runs (about 2 Gb raw data each species). De novo assemblies were conducted with Geneious 9.1.4 using the Map to Reference program (Kearse et al. 2012) with parameter settings (Minimum Overlap = 30~50, Minimum Overlap Identity: 80~100, Maximum Mismatches Per Read = 10%). The mitochondrial contigs were then extracted and identified using BLAST alignment against the closest ref-

erence mitogenome at NCBI. Sanger sequencing (Sanger and Coulson 1975) was also used to verify the gene fragments and to obtain fragments between two long contigs where some reads were not assembled.

Mitogenome annotation and analyses

The assembled and identified mitochondrial DNA sequences were further annotated and analyzed. The locations of PCGs and rRNA genes were preliminarily annotated by DOGMA website (Wyman et al. 2004). The coding regions of PCGs were identified by using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/), and subsequently annotated by alignments of homologous genes of other published brachyuran mitogenomes. Codon usage of PCGs was determined by MEGA 6 (Tamura et al. 2013). Transfer RNA genes were mainly identified by tRNAscan-SE (Lowe and Eddy 1997); the remains were identified according to their tRNA-like secondary structures and anticodon sequences. Two rRNA genes were identified by alignment with other published brachyuran sequences. The nucleotide sequences of the complete mitogenomes for *S. yaanense* and *S. yangtsekiense* were deposited in the NCBI database under the Accession No. KY785880 and KY785879.

Phylogenetic analysis

In-group and out-group taxa are listed in Table 1. After removing all termination codons, the putative amino acid (AA) sequences for each of the 13 mt PCGs and sequences for two rRNAs were individually aligned using MAFFT 7.215 (Katoh and Standley 2013) with the iterative refinement method G-INS-I (accurate alignment), in which the gap opening and extension penalties were 1.53 and 0.123, respectively. Ambiguous or randomly similar sites were removed by Aliscore 2.0 (Misof and Misof 2009; Kuck et al. 2010) using default settings. These AA alignments were later used as a backbone to align the corresponding nucleotide (NT) sequences using DAMBE 5.3.15 (Xia and Xie 2001). All 13 PCGs alignments were combined to create dataset A, and two rRNAs and 13 PCGs were concatenated to create dataset B. Subsequently, PartitionFinder 1.1.1 (Lanfear et al. 2012) was used to find the best-fit partitioning schemes and models using a greedy search with RAxML (Stamatakis 2006a) and Bayesian information criterion (BIC). Maximum likelihood (ML) analyses for all datasets were performed using RAxML-HPC2 on XSEDE 8.0.0 (Stamatakis 2014) through the Cipres Science Gateway (Miller et al. 2010). Given the advantages of Gamma over CAT when the sequences were less than 50 (Stamatakis 2006b) and the best-fit models calculated by the PartitionFinder 1.1.1 was not recommended by the developer of RAxML (Mayrose et al. 2005; Stamatakis 2006a), the model GTR + Gamma for all NT datasets were used. MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001) was used to perform Bayesian inference (BI) analysis.

Table 1. Taxon sampled and its mitogenomic information used in this study.

	Taxon	Species	Length (bp)	GenBank Accession No.	Reference
Ingroup					
Brachyura	**Podotremata**				
	Homoloidea	*Homologenus malayensis*	15793	KJ612407	Unpublished
		Moloha majora	15903	KT182069	Shi et al. 2016
	Raninoidea				
		Umalia orientalis	15466	NC_026688	Shi et al. 2015
		Ranina ranina	15557	NC_023474	unpublished
		Lyreidus brevifrons	16112	NC_026721	Shi et al. 2015
Eubrachyura					
Heterotremata					
Portunoidea					
		Thalamita crenata	15787	NC_024438	Tan et al. 2016a
		Callinectes sapidus	16263	NC_006281	Place et al. 2005
		Portunus trituberculatus	16026	NC_005037	Yamauchi et al. 2003
		Charybdis japonica	15738	NC_013246	Liu et al. 2010
		Chaceon granulatus	16135	NC_023476	unpublished
		Scylla olivacea	15723	NC_012569	unpublished
	Majoidea				
		Chionoecetes japonicus	15341	AB_735678	unpublished
Calappoidea					
		Ashtoret lunaris	15807	NC_024435	Tan et al. 2016b
	Eriphioidea				
		Myomenippe fornasinii	15658	NC_024437	Tan et al. 2016c
	Xanthoidea				
		Pseudocarcinus gigas	15515	NC_006891	Miller et al. 2005
	Bythograeoidea				
		Gandalfus yunohana	15567	NC_013713	Yang et al. 2010
		Austinograea alayseae	15620	NC_020314	Yang et al. 2013
	Potamoidea				
		Geothelphusa dehaani	18197	NC_007379	Segawa et al. 2005
		Sinopotamon xiushuiense	18460	NC_029226	Wang et al. 2016
		S. yangtsekiense	17885	KY785879	Present study
		S. yaanense	17126	KY785880	Present study
Thoracotremata					
Ocypodoidea					
		Ilyoplax deschampsi	15460	NC_020040	Ji et al. 2014
		Ocypode ceratophthalmus	15564	NC_025324	Tan et al. 2016e
		Mictyris longicarpus	15548	LN_611670	Tan et al. 2016d
Grapsoidea					
		Eriocheir japonica sinensis	16354	NC_006992	Sun et al. 2005
		Xenograpsus testudinatus	15798	NC_013480	Ki et al. 2009
		Pachygrapsus crassipes	15652	NC_021754	Yu et al. 2014
		Cyclograpsus granulosus	16300	NC_025571	Tan et al. 2016f
		Chiromantes neglectum	15920	KX156954	Xing et al. 2016

		Parasesarma tripectinis	15612	KU343209	Unpublished
		Sesarmops sinensis	15905	KR336554	Unpublished
		Metopaulias depressus	15765	KX118277	Unpublished
Outgroup					
Anomura	**Paguroidea**				
		Pagurus longicarpus	15630	NC_003058	Hickerson et al. 2000
		Paralithodes brevipes	16303	NC_021458	Unpublished
		Lithodes nintokuae	15731	NC_024202	Unpublished
	Galatheoidea	*Neopetrolisthes maculatus*	15324	NC_020024	Shen et al. 2013
		Shinkaia crosnieri	15182	NC_011013	Yang et al. 2008
Thalassinidea	**Gebiidea**				
		Austinogebia edulis	15761	NC_019606	Lin et al. 2012
		Upogebia major	16119	JF793665	Kim et al. 2011a
Polychelidae	**Eryonoidea**				
		Polycheles typhlops	16221	NC_020026	Shen et al. 2013
Astacidea	**Astacoidea**				
		Cambaroides similis	16220	NC_016925	Kim et al. 2012
	Nephropoidea				
		Homarus americanus	16432	NC_015607	Kim et al. 2011b
	Parastacoidea				
		Cherax destructor	15894	AY383557	Miller et al. 2004

Results

Mitogenome size and organization

The complete mitogenome sequences of *S. yaanense* and *S. yangtsekiense* were, respectively, 17,126 bp and 17,885 bp in length, between 15,612 bp and 18,460 bp typical in length for eubrachyurans (Table 1). Both sequences contained the entire set of 37 genes plus a mNCR (Fig. 1). The larger mitogenome size in *S. yangtsekiense* was due to greatly expanded non-coding nucleotides of intergenic spacers with 1,964 bp in total (ranging from 1 to 656 bp), but only 1,135 bp in *S. yaanense* (from 1 to 321 bp).

A non-coding region between *rrnS* and *trnI* was identified as the main non-coding region (mNCR, or AT-rich region) for the two *Sinopotamon* crab species. The mNCR consisted of 1231 bp in *S. yaanense* (with 78.9% A + T content), whereas it consisted of 1194 bp in *S. yangtsekiense* (79.3%). The alignment of the entire sequences of mNCR revealed a high similarity between the two *Sinopotamon* species in two dormains (Suppl. material 1), characterized by a highly conserved central domain and extended termination associated sequences (ETAS).

The overall A + T content of 13 PCGs, calculated from the coding strand of each gene, was 70.7% in *S. yaanense* and 71.8% in *S. yangtsekiense* (Table 2). *Cox1* had the lowest A + T content (64.8%) and *nad4* the highest (74.5%) in *S. yaanense*, whereas for *S. yangtsekiense cox1* the lowest (65.6%) and *nad6* the highest (77.0%). Of the 13
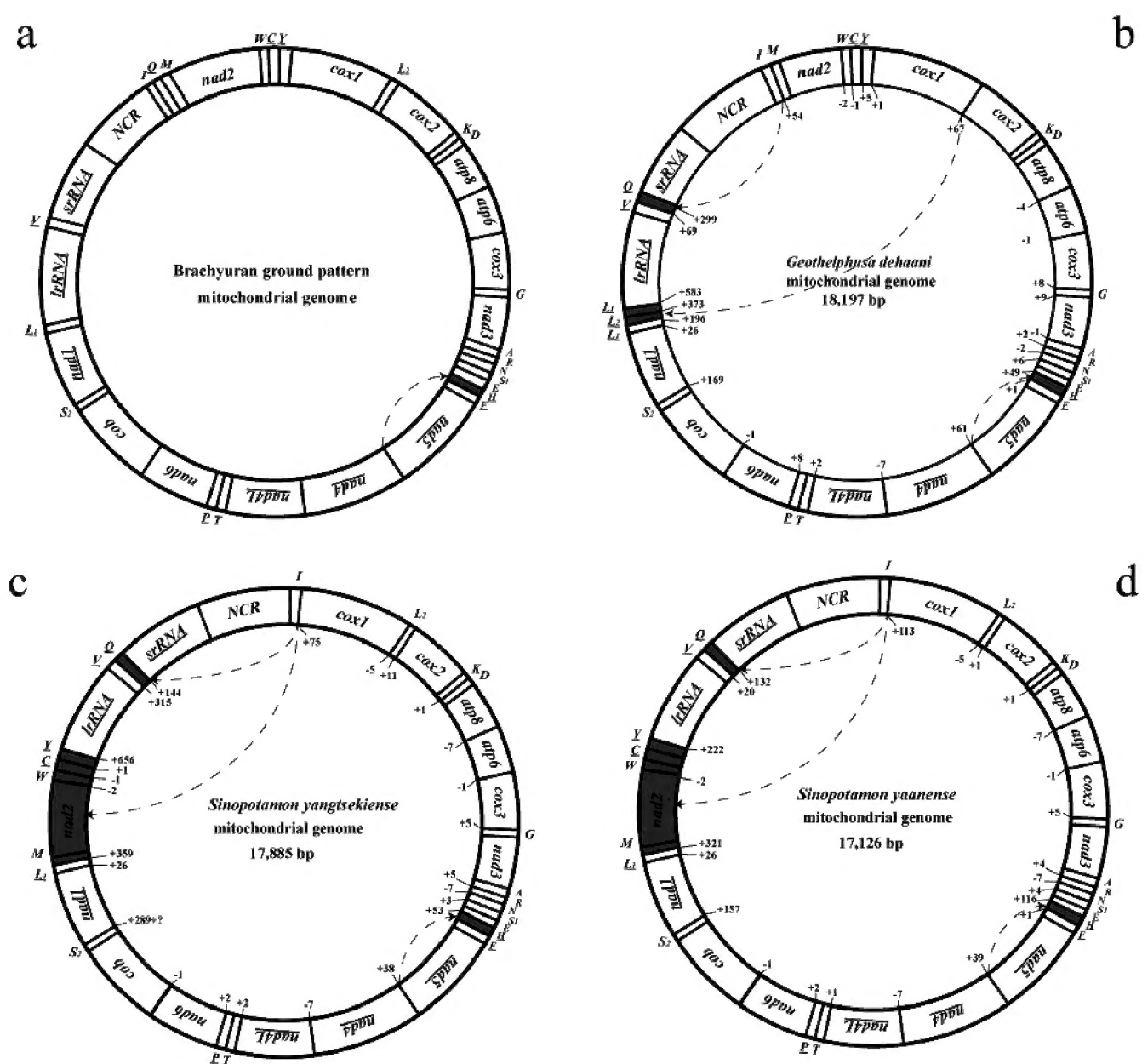
Figure 1. Mitochondrial genome sequenced in the present study. Gene order and sizes are shown relative to one another, including non-coding regions. Protein-coding genes encoded on the light strand are underlined. Transfer RNA (tRNA) genes encoded on the light strand are underlined. Each tRNA gene is designated by a single-letter amino acid code, except *L1 (trnLeu (CUN)), L2 (trnLeu (UUR)), S1 (trnSer (AGN))* and *S2 (trnSer (UCN))*. Numbers inside circles represent the size of the non-coding region separating two adjacent genes or the amount of shared nucleotides between two overlapping genes. The translocations of gene or gene block are shaded gray.

PCGs, nine genes started with the initiation codons ATG (*cox1, cox2, atp8, cox3, nad4, nad4L, nad5, cob* and *nad2*); the others began with ATA, ATC and ATT (*atp6, nad3, nad6* and *nad1*) (Table 2). Meanwhile, ten genes used the typical termination codons TAG and TAA (*cox1, atp6, atp8, cox3, nad3, nad4, nad4L, cob, nad1* and *nad2*); the remaining genes used incomplete stop codons T (*cox2, nad5* and *cob*) (Table 2).

Gene rearrangement

Gene orders of *S. yaanense* and *S. yangtsekiense* mitogenomes were identical between both species (Fig. 1). Compared to the general brachyuran pattern, a novel gene order

Table 2. Nucleptide composition in 13 PCGs of the *Sinopotamon yaanense* and *S. yangtsekiense* mitogenomes.

	Species	Base composition(%)				A+T (%)	Length (nt)	Start codons	Stop codons
		A	T	G	C				
cox1	S. yaanense	27.60	37.20	15.70	19.50	64.80	1539	ATG	TAA
	S. yangtsekiense	27.70	37.90	16.00	18.30	65.60	1539	ATG	TAA
cox2	S. yaanense	31.88	37.26	11.79	19.07	69.14	688	ATG	T
	S. yangtsekiense	31.43	39.62	12.13	16.81	71.05	685	ATG	T
atp8	S. yaanense	26.14	47.71	7.19	18.95	73.86	159	ATG	TAG
	S. yangtsekiense	27.45	49.02	4.58	18.95	76.47	159	ATG	TAG
atp6	S. yaanense	30.06	40.63	10.42	18.90	70.68	675	ATT	TAA
	S. yangtsekiense	29.76	41.52	10.71	18.01	71.28	675	ATT	TAA
cox3	S. yaanense	26.62	39.67	14.45	19.26	66.29	792	ATG	TAA
	S. yangtsekiense	27.25	40.43	13.81	18.50	67.68	792	ATG	TAA
nad3	S. yaanense	27.64	44.73	11.68	15.95	72.36	354	ATC	TAG
	S. yangtsekiense	27.64	44.44	11.11	16.81	72.08	354	ATC	TAG
nad5	S. yaanense	30.84	41.61	18.75	8.80	72.45	1729	ATG	T
	S. yangtsekiense	30.84	41.61	18.75	8.80	72.45	1729	ATG	T
nad4	S. yaanense	32.35	42.19	17.36	8.10	74.54	1335	ATG	TAG
	S. yangtsekiense	30.94	42.92	18.35	7.79	73.86	1338	ATG	TAA
nad4L	S. yaanense	26.00	46.33	22.00	5.67	72.33	303	ATG	TAA
	S. yangtsekiense	27.00	47.33	20.33	5.33	74.33	303	ATG	TAA
nad6	S. yaanense	26.91	47.39	6.63	19.08	74.30	504	ATT	TAA
	S. yangtsekiense	27.38	49.60	6.55	16.47	76.98	507	ATT	TAA
cob	S. yaanense	27.43	40.39	12.35	19.84	67.81	1135	ATG	T
	S. yangtsekiense	27.43	41.53	12.70	18.34	68.96	1135	ATG	T
nad1	S. yaanense	26.82	45.09	19.23	8.87	71.90	939	ATA	TAA
	S. yangtsekiense	27.67	45.09	18.48	8.76	72.76	939	ATA	TAA
nad2	S. yaanense	28.44	45.41	7.49	18.66	73.85	1005	ATG	TAA
	S. yangtsekiense	29.24	46.11	7.58	17.07	75.35	1005	ATG	TAA
total	S. yaanense	28.36	42.74	13.46	15.43	70.67	11157		
	S. yangtsekiense	28.59	43.63	13.16	14.61	71.81	11160		

was found for both *Sinopotamon* crab species with translocations of a gene block and a tRNA gene, where the major rearrangement was due to a five-gene block (*trnM-nad2-trnW-trnC-trnY*) translocated out of *trnI-cox1* gene junction and moved into *trnL1-lrRNA* gene junction (i.e. *trnL1-* **trnM-nad2-trnW-trnC-trnY***-lrRNA*). The minor rearrangement was attributed to a transposition of *trnQ* that occurred in mitogenome sequences available for potamid crabs (Fig. 2) that moved from a tRNA gene cluster (*I-Q-M*) to *trnV* and *srRNA* gene junction (i.e. *trnV-***trnQ***-srRNA*).

Phylogenetic inference

The phylogenetic trees were reconstructed based on the two different datasets A (13 PCGs) and B (13 PCGs + two rRNAs). Both ML and Bayesian analyses re-
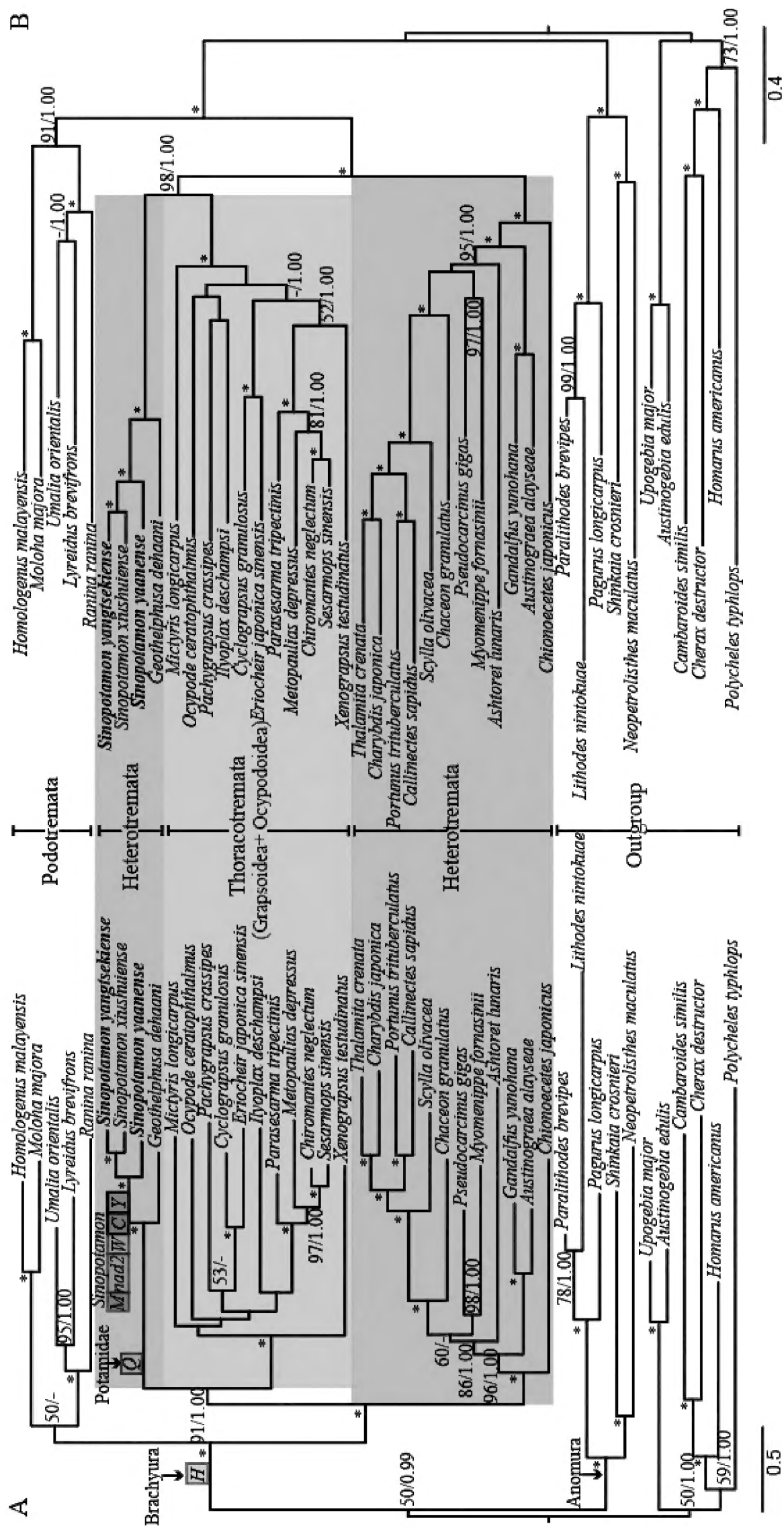
Figure 2. Phylogenetic analyses derived for brachyurans using the maximum likelihood (ML) analyses and Bayesian inferences (BI) using dataset A (13 PCGs) and dataset B (13 PCGs + two rRNAs). Branch lengths and topologies came from ML analysis. Values at the branches represent BP (Bootstrap value)/BPP (Bayesian posterior probability). 100/1.00 is denoted by an asterisk. The horizontal line stands for BP under 50 or BPP under 0.9 ML analyses. The gene rearrangement is denoted by the block on (A): (I) the translocation of *trnH* shared by the Brachyura taxa sampled; (II) the transposition of *trnQ* shared by potamid species; (III) the five-gene block, (*trnM-nad2-trnW-trnC-trnY*), translocation shared by three *Sinopotamon* crabs sampled.

sulted in congruent tree topologies with the exception of some minor difference within "Grapsoidea + Ocypodoidea". Branch lengths and topologies came from ML analysis. Bootstrap value (BP) and Bayesian posterior probability (BPP) of nodes are shown like BP/BPP in Fig. 2. The Brachyura and Anomura clades grouped together, recovered as a monophyletic Meiura group, but with strong support in only one Bayesian analysis. The homolid crabs and raninid crabs within the Brachyura formed a monophyletic Podotremata clade with strong support from dataset B analysis (BP = 91; BPP = 1.00), but weakly supported by dataset A analysis (BP =50; BPP < 0.9). All trees place the Podotremata clade as a sister group of the eubrachyuran clade with relatively high nodal support. These results provided evidence for the monophyly of Brachyura and Eubrachyura.

Within the Eubrachyura, strong support was obtained for the non-monophyly of Heterotremata, with potamid crabs nesting outside the heterotreme clade ((((Portunoidea + Calappoidea) + (Xanthoidea + Eriphioidea)) + Bythograeoidea) + Majoidea); instead, four potamid crabs appeared to constitute a close affinity to Thoracotremata, and form a robust clade ((Grapsoidea + Ocypodoidea)+Potamidae). All potamids, *S. yaanense*, *S. yangtsekiense* and *S. xiushuiense* constituted a robust clade with *G. dehaani* being sister to this clade in all analyses, with relatively high nodal support (BP = 100 and 100; BPP = 1.00 and 1.00).

Discussion

The mitogenomic organizations found in the two *Sinopotamon* species are identical to those of the typical decapods (Qian et al. 2011). The A + T content of all 13 PCGs in the two species were obtained, indicating an AT bias (70.7% in *S. yaanense*, and 71.8% in *S. yangtsekiense*) similar to that reported in most of the mitogenomes available for brachyurans (Bai et al. 2016). This high A + T content may be correlated with the strong A + T bias in codon usage (Song et al. 2016; Tan et al. 2017). The initiation codons of 13 PCGs are usual ATN, but the incomplete stop codon T is assigned for three PCGs (Table 2). These variable start codons and incomplete stop codons (T and/or TA) are frequently found in other brachyurans (Yamauchi et al. 2003; Sun et al. 2005; Shi et al. 2015; Bai et al. 2016), and may be completed by polyadenylation during the mRNA maturation process (Ojala et al. 1981; Qiu et al. 2005).

The largest non-coding region between *rrnS* and *trnI* was predicted to be the putative mNCR in the two *Sinopotamon* crab species ranging from 1194 bp (*S. yangtsekiense*) to 1231 bp (*S. yaanense*), where the position is similar to that found in *Geothelphusa dehaani* and other brachyurans (see Segawa and Aotsuka 2005; Bai et al. 2016; Ma et al. 2016a, 2016b). The comparisons of mNCR of the two *Sinopotamon* species revealed a highly conserved structure, including a central domain and ETAS (Supporting information, Suppl. material 1). The similarity of nucleotide composition between the two species is 77% and 70% in these two domains, respectively. A similar characterized structure has been demonstrated in vertebrates (Saccone et al. 1987; Sbisa et al.

1997; Kierstein et al. 2004). The similar structure can be also identified from the largest intergenic spacer consisting of 1221 bp for *S. xiushuiense* mitogenome, although this intergenic spacer was not regarded as the mNCR by Wang et al. (2016). Furthermore, several repeated motifs were identified at the 3' end of the central domain for three *Sinopotamon* species, including TA (TC), TA (T), CA (TA) and AT (AA). Similar motifs were found in other brachyurans (Pie et al. 2008; Jondeung et al. 2012; Shi et al. 2015; Ma et al. 2016a, 2016b). These results indicate that mNCR could be used as a genetic marker for studies regarding population structure and phylogeographic patterns of related species (Diniz et al. 2005).

Gene arrangements and primary sequences in mitogenomes embrace useful signals in reconstruction of phylogenetic relationships (Boore et al. 1995, 1998; Boore 1999; Sun et al. 2003, 2005; Tan et al. 2017). The brachyuran crabs are among the most specious groups in decapod crustaceans (Ng et al. 2008; Guinot et al. 2013). In terms of gene rearrangement, our results revealed that brachyuran crabs share a translocation of *trnH*, which moved from the *nad5-nad4* gene junction into a major tRNA gene cluster (*A-R-N-S1-E-F*) between the *nad3* and *nad5* genes; this is common for brachyuran mitogenomes (Fig. 2; Yamauchi et al. 2003; Sun et al. 2005; Shi et al. 2015). A translocation of tRNA gene in mitogenome, even if this is minor rearrangement, provides in several cases convincing phylogenetic evidence of deducing phylogenetic relationships among major groups of arthropods (Boore et al. 1995, 1998). The shared rearrangement of *trnH* in brachyurans agrees with the molecular phylogeny presented here and previous studies, which supports the monophyly of Brachyura (Jamieson et al. 1994, 1995; Scholtz and McLay 2009; Qian et al. 2011; van Bakel et al. 2012; Ji et al. 2014; Tsang et al. 2014; Shi et al. 2015). Compared to the ancestral pattern of mitochondrial gene order of brachyurans, we further found that a translocation of *trnQ* was shared by mitogenomes available for the sampled potamids (Fig. 2). Furthermore, a novel rearrangement involving five genes (*trnM-nad2-trnW-trnC-trnY*) was only found in mitogenomes available for *Sinopotamon* species. Common gene rearrangements that possibly act as synapomorphy for phylogenetic estimation have been identified in decapod crustaceans (Shen et al. 2015; Tan et al. 2015, 2017). Here we found that novel gene order attributes act as useful phylogenetic characters for Potamidae. We propose that *trnQ* rearrangement is most likely a synapomorphy in potamid lineages, and the distinct five gene block rearrangement might be a derived pattern for *Sinopotamon* crabs during the long period of evolution of potamid lineages. More gene rearrangement patterns and their evolutionary implications in potamids may be better understood through the comparison of mitogenomes of a broader taxon sampling.

Striking species diversification has challenged the phylogeny of brachyurans and led to a high number of hypotheses about affinities within the Eubrechyura (reviewed in Guinot et al. 2013; Tsang et al. 2014). All primary freshwater crabs, involving potamids, are recognized as true heterotremes (Guinot et al. 2013). The monophyletic nature of Potamidae is confirmed by morphological and molecular analyses (reviewed in Yeo et al. 2008). However, its phylogenetic placement remains controversial based on morphological characters and molecular phylogeny (Dai 1999; Von Sternberg et al.

1999; Von Sternberg and Cumberlidge 2001). Our results show that these heterotreme crabs, *G. dehaani*, *S. xiushuiense*, *S. yaanense* and *S. yangtsekiense* are in fact associated more closely with thoracotreme crabs than to the remaining heterotremes. Therefore, we propose a phylogenetic framework for the non-monophyly of Heterotremata. However, in the combined gene tree presented by Tsang et al. (2014), the potamid clade along with the other primary freshwater crabs align with heterotreme crabs rather than with thoracotreme crabs (without strong support). Interrelationships of major clades within the Eubrachyura remain one of the most contentious issues in systematics today. Von Sternberg and Cumberlidge (2001) suggested that families of Old World freshwater crabs including Potamonautidae endemic to the Afrotropical region, the Potamidae and Gecarcinucidae endemic to Oriental region, should be placed in Thoracotremata and aligned with gecarcinids, grapsoids and ocypodoids s.l. based on inferences of multiple adult morphological characters. Recent molecular phylogenetic analyses based on DNA also suggested that the Heterotremata is non-monophyletic (Schubart et al. 2000; Porter et al. 2005; Ahyong et al. 2007; Mahon and Neigel 2008; Bracken et al. 2009; Ji et al. 2014; Xing et al. 2016). In this context, rapid and efficient methods using next-generation sequencing could recover sufficient mitogenomes through far greater taxon sampling and build a high-resolution phylogenetic relationship within the Brachyura.

Acknowledgements

References

Ahyong ST, Lai JC, Sharkey D, Colgan D, Ng PKL (2007) Phylogenetics of the brachyuran crabs (Crustacea: Decapoda): the status of Podotremata based on small subunit nuclear ribosomal RNA. Molecular Phylogenetics and Evolution 45: 576–586. https://doi.org/10.1016/j.ympev.2007.03.022

Bai J, Zhu CC, Nie Z, Zou JX, Wang Y, Zhou XM (2016) Introduction and comparative analysis of brachyura mitochondrial genomes research. Genomics and Applied Biology 35(10): 2627–2639. https://doi.org/10.13417/j.gab.035.002627

Boore JL, Collins TM, Stanton D, Daehler LL, Brown WM (1995) Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature 376(6536): 163–165. https://doi.org/10.1038/376163a0

Boore JL, Lavrov DV, Brown WM (1998) Gene translocation links insects and crustaceans. Nature 392: 667-668. https://doi.org/10.1038/33577

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Research 27(8): 1767–1780. https://doi.org/10.1093/nar/27.8.1767

Bracken HD, Toon A, Felder DL, Martin JW, Finley M, Rasmussen J, Palero F, Crandall KA (2009) The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. Arthropod Systematics and Phylogeny 67: 99–116. www.arthropod-systematics.de on 17.vi.2009.

Castro P, Davie PJF, Guinot D, Schram FR, von Vaupel Klein JC (2015) The Crustacea; complementary to the volumes translated from the French of the Traite de Zoologie. Brill, Leiden Boston, 163 pp.

Cumberlidge N, von Sternberg RM, Daniels SR (2008) A revision of the higher taxonomy of the Afrotropical freshwater crabs (Decapoda: Brachyura) with a discussion of their biogeography. Biological Journal of the Linnean Society 93: 399–413. https://doi.org/10.1111/j.1095-8312.2007.00929.x

Dai AY (1999) Fauna Sinica. Arthropoda: Crustacea: Malacostraca: Decapoda: Parathelphusidae, Potamidae. Science Press, Beijing, 501 pp.

de Saint-Laurent M (1980) Sur la classification et la phylogénie des Crustacés Décapodes Brachyoures. I. Podotremata Guinot, 1977, et Eubrachyura sect. nov. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences Pairs (D) 290: 1265–1268. https://www.scienceopen.com/document?vid=a6905901-13e3-4522-b227-b152f1e7aad6

Diniz FM, Maclean N, Ogawa M, Cintra IH, Bentzen P (2005) The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. Marine Biotechnology 7(5): 462–473. https://doi.org/10.1007/s10126-004-4062-5

Fang F, Ji YK, Zhao Q, Wang YJ, Gao W, Chu KL, Sun HY (2015) Phylogeography of the Chinese endemic freshwater crab *Sinopotamon acutum* (Brachyura, Potamidae). Zoologica Scripta 44: 653–666. https://doi.org/10.1111/zsc.12131

Fang F, Sun HY, Zhao Q, Lin CT, Sun YF, Gao W, Xu JJ, Zhou JY, Ge F, Liu NF (2013) Patterns of diversity, areas of endemism, and multiple glacial refuges for freshwater crabs of the genus *Sinopotamon* in china (Decapoda: Brachyura: Potamidae). PLoS ONE 8: e53143. https://doi.org/10.1371/journal.pone.0053143

Gan HM, Schultz MB, Austin CM (2014a) Integrated shotgun sequencing and bioinformatics pipeline allows ultra-fast mitogenome recovery and confirms substantial gene rearrangements in Australian freshwater crayfishes. BMC Evolutionary Biology 14: 19. https://doi.org/10.1186/1471-2148-14-19

Gan HM, Tan MH, Austin CM (2014b) The complete mitogenome of the Australian spiny crayfish *Euastacus yarraensis* (McCoy, 1888) (Crustacea: Decapoda: Parastacidae). Mitochondrial DNA 27: 929–930. https://doi.org/10.3109/19401736.2014.926490

Gan HM, Tan MH, Austin CM (2014c) The complete mitogenome of the red claw crayfish *Cherax quadricarinatus* (Von Martens, 1868) (Crustacea: Decapoda: Parastacidae). Mitochondrial DNA 27: 385–386. https://doi.org/10.3109/19401736.2014.895997

Gan HM, Tan MH, Eprilurahman R, Austin CM (2014d) The complete mitogenome of *Cherax monticola* (Crustacea: Decapoda: Parastacidae), a large highland crayfish from New Guinea. Mitochondrial DNA 27: 337–338. https://doi.org/10.3109/19401736.2014.892105

Gan HM, Tan MH, Gan HY, Lee YP, Schultz MB, Austin CM (2014e) The complete mitogenome of the Australian crayfish *Geocharax gracilis* Clark 1936 (Crustacea: Decapoda: Parastacidae). Mitochondrial DNA 27: 826–827. https://doi.org/10.3109/19401736.2014.919460

Grabherr MG, Haas BJ, Yassour M (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29: 644–652. https://doi.org/10.1038/nbt.1883

Guinot D (1977) Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences 285: 1049–1052.

Guinot D, Tavares M, Castro P (2013) Significance of the sexual openings and supplementary structures on the phylogeny of brachyuran crabs (Crustacea, Decapoda, Brachyura), with new nomina for higher-ranked podotreme taxa. Zootaxa 3665(1): 1–414. https://dx.doi.org/10.11646/zootaxa.3665.1.1

Hickerson MJ, Cunningham CW (2000) Dramatic mitochondrial gene rearrangements in the hermit crab *Pagurus longicarpus* (Crustacea, Anomura). Molecular Biology and Evolution 17(4): 639–644. https://doi.org/10.1093/oxfordjournals.molbev.a026342

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Jamieson BGM (1994) Phylogeny of the Brachyura with particular reference to the Podotremata: evidence from a review of spermatozoal ultrastructure (Crustacea, Decapoda). Philosophical Transactions of the Royal Society of London B: Biological Sciences 345(1314): 373–393. https://doi.org/10.1098/rstb.1994.0116

Jamieson BGM, Guinot D, Richer de Forges B (1995) Phylogeny of the Brachyura (Crustacea, Decapoda): evidence from spermatozoal ultrastructure. In: Jamieson BGM, Ausio J, Justine JL (Eds) Advances in Spermatozoal Phylogeny and Taxonomy. Mémoires du Muséum national d'Histoire naturelle 166: 265–283.

Jex AR, Hall RS, Littlewood DT, Gasser RB (2010) An integrated pipeline for next-generation sequencing and annotation of mitochondrial genomes. Nucleic Acids Research 38(2): 522–533. https://doi.org/10.1093/nar/gkp883

Ji YK, Wang A, Lu JJ, Lu XL, Jin YH, Song DH, Sun HY (2014) Mitochondrial genomes of two brachyuran crabs (Crustacea: Decapoda) and phylogenetic analysis. Journal of Crustacean Biology 34: 494–503. https://doi.org/10.1163/1937240X-00002252

Ji YK, Sun YH, Gao W, Chu KL, Wang R, Zhao Q, Sun HY (2016) Out of the Sichuan Basin: Rapid species diversification of the freshwater crabs in *Sinopotamon* (Decapoda: Brachyura: Potamidae) endemic to China. Molecular Phylogenetics and Evolution 100: 80–94. https://doi.org/10.1016/j.ympev.2016.04.003

Jondeung A, Karinthanyakit W, Kaewkhumsan J (2012) The complete mitochondrial genome of the black mud crab, *Scylla serrata* (Crustacea: Brachyura: Portunidae) and its phylogenetic position among (pan) crustaceans. Molecular Biology Reports 39(12): 10921–10937. https://doi.org/10.1007/s11033-012-1992-2

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. doi:10.1093/molbev/mst010

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C (2012) GENEIOUS Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Ki JS, Dahms HU, Hwang JS, Lee JS (2009) The complete mitogenome of the hydrothermal vent crab *Xenograpsus testudinatus* (Decapoda, Brachyura) and comparison with brachyuran crabs. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 4(4): 290–299. https://doi.org/10.1016/j.cbd.2009.07.002

Kierstein G, Vallinoto M, Silva A, Schneider MP, Iannuzzi L, Brenig B (2004) Analysis of mitochondrial D-loop region casts new light on domestic water buffalo (*Bubalus bubalis*) phylogeny. Molecular Phylogenetics and Evolution 30: 308–324. https://doi.org/10.1016/S1055-7903(03)00221-5

Kim S, Kim T, Choi HG, Park JK, Ahn DH, Min GS (2011a) The complete mitochondrial genome of the Japanese mud shrimp *Upogebia major* (Crustacea, Decapoda). Mitochondrial DNA 22(4): 94–96. https://doi.org/10.1186/1471-2164-13-631

Kim S, Lee SH, Park MH, Choi HG, Park JK, Min GS (2011b) The complete mitochondrial genome of the American lobster, *Homarus americanus* (Crustacea, Decapoda). Mitochondrial DNA 22(3): 47–49. https://doi.org/10.3109/19401736.2011.597389

Kim S, Park MH, Jung JH, Ahn DH, Sultana T, Kim S, Min GS (2012) The mitochondrial genomes of *Cambaroides similis* and *Procambarus clarkii* (Decapoda: Astacidea: Cambaridae): the phylogenetic implications for Reptantia. Zoologica Scripta 41(3): 281–292. https://doi.org/10.1111/j.1463-6409.2012

Kuck P, Meusemann K, Dambach J, Thormann B, von Reumont BM, Wägele JW, Misof B (2010) Parametric and non-parametric masking of randomness in sequence alignments can be improved and leads to better resolved trees. Frontiers in Zoology 7: 10. https://doi.org/10.1186/1742-9994-7-10

Lanfear R, Calcott B, Ho SY, Guindon S (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Liu Y, Cui Z (2010) Complete mitochondrial genome of the Asian paddle crab *Charybdis japonica* (Crustacea: Decapoda: Portunidae): gene rearrangement of the marine brachyurans and phylogenetic considerations of the decapods. Molecular Biology Reports 37(5): 2559–2569. https://doi.org/10.1007/s11033-009-9773-2

Lin FJ, Liu Y, Sha Z, Tsang LM, Chu KH, Chan TY, Cui Z (2012) Evolution and phylogeny of the mud shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes. BMC Genomics 13(1): 631. https://doi.org/10.1186/1471-2164-13-631

Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Research 25: 955–964. https://doi.org/10.1093/nar/25.5.0955

Ma CY, Ma HY, Ren GJ, Wang W, Chen W, Lu JX (2016a) Characterization of the complete mitochondrial genome of *Portunus pelagicus* with implications for phylogenomics.

Genetics and Molecular Research 15(3): gmr.15038719. http://dx.doi.org/10.4238/gmr.15038719

Ma HY, Ma CY, Zhu JJ, Ren GJ, Wang W, Chen W (2016b) Characterization of the complete mitochondrial genome and phylogenetic relationships of the three-spot swimming crab (*Portunus sanguinolentus*). Genetics and Molecular Research 15(3): gmr.15038580. http://dx.doi.org/10.4238/gmr.15038580

Mahon BC, Neigel JE (2008) Utility of arginine kinase for resolution of phylogenetic relationships among brachyuran genera and families. Molecular Phylogenetics and Evolution 48: 718–727. https://doi.org/10.1016/j.ympev.2008.05.005

Mayrose I, Friedman N, Pupko T (2005) A Gamma mixture model better accounts for among site rate heterogeneity. Bioinformatics 21: 151–158. https://doi.org/10.1093/bioinformatics/bti1125

Miller AD, Murphy NP, Burridge CP, Austin CM (2005) Complete mitochondrial DNA sequences of the decapod crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii* (Palaemonidae). Marine Biotechnology 7(4): 339–349. https://doi.org/10.1007/s10126-004-4077-8

Miller AD, Nguyen TT, Burridge CP, Austin CM (2004) Complete mitochondrial DNA sequence of the Australian freshwater crayfish, *Cherax destructor* (Crustacea: Decapoda: Parastacidae): a novel gene order revealed. Gene 331: 65–72. https://doi.org/10.1016/j.gene.2004.01.022

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE) IEEE 2010: 1–8. https://doi.org/10.1109/GCE.2010.5676129

Misof B, Misof K (2009) A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. Systematic Biology 58: 21–34. https://doi.org/10.1093/sysbio/syp006

Ng PKL, Guinot D, Davie PJF (2008) Systema Brachyuorum: Part I. An annotated checklist of extant brachyuran crabs of the world. Raffles Bulletin of Zoology 17: 1–286. http://www.vliz.be/imisdocs/publications/133883.pdf

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290: 470–474. https://doi.org/10.1038/290470a0

Pie MR, Oliveira-Neto JF, Boeger WA, Ostrensky A, Baggio RA (2008) The organization of the mitochondrial control region in 2 brachyuran crustaceans: *Ucides cordatus* (Ocypodidae) and *Cardisoma guanhumi* (Gecarcinidae). Journal of Heredity 99(4): 432–437. doi:10.1093/jhered/esn024

Place AR, Feng X, Steven CR, Fourcade HM, Boore JL (2005) Genetic markers in blue crabs (*Callinectes sapidus*): II. Complete mitochondrial genome sequence and characterization of genetic variation. Journal of Experimental Marine Biology and Ecology 319(1): 15–27. https://doi.org/10.1016/j.jembe.2004.03.024

Porter ML, Perez-Losada M, Crandall KA (2005) Model-based multi-locus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution 37: 355–369. https://doi.org/10.1016/j.ympev.2005.06.021

Qian GH, Zhao Q, Wang A, Zhu L, Zhou KY, Sun HY (2011) Two new decapod (Crustacea: Malacostraca) complete mitochondrial genomes: bearings on the phylogenetic relationships within the Decapoda. Zoological Journal of the Linnean Society 162: 471–481. https://doi.org/10.1111/j.1096-3642.2010.00686.x

Qiu Y, Song DX, Zhou KY, Sun HY (2005) The mitochondrial sequences of *Heptathela hangzhouensis* and *Ornithoctonus huwena* reveal unique gene arrangements and atypical tRNAs. Journal of Molecular Evolution 60: 57–71. https://doi.org/10.1007/s00239-004-0010-2

Saccone C, Attimonelli M, Sbisa E (1987) Structural elements highly preserved during the evolution of the D-loop-containing region in vertebrate mitochondrial DNA. Journal of Molecular Evolution 26: 205–211. https://doi.org/10.1007/BF02099853

Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology 94(3): 441–446. https://doi.org/10.1016/0022-2836(75)90213-2

Sbisa E, Tanzariello F, Reyes A, Pesole G, Saccone C (1997) Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene 205: 125–140. http://dx.doi.org/10.1016/S0378-1119(97)00404-6

Scholtz G, McLay CL (2009) Is the Brachyura Podotremata a monophyletic group? Crustaceana 18: 417–435. https://doi.org/10.1201/9781420092592-c21

Schubart CD, Neigle J E, Felder DL (2000) Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. Crustacean Issues 12: 817–830. http://www.biologie.uni-regensburg.de/Zoologie/Heinze/Staff/ChristophSchubart/pdf/CrustIss-2000errata.pdf

Segawa RD, Aotsuka T (2005) The mitochondrial genome of the Japanese freshwater crab, *Geothelphusa dehaani* (Crustacea: Brachyura): evidence for its evolution via gene duplication. Gene 355: 28–39. https://doi.org/10.1016/j.gene.2005.05.020

Shen H, Braband A, Scholtz G (2013) Mitogenomic analysis of decapod crustacean phylogeny corroborates traditional views on their relationships. Molecular Phylogenetics and Evolution 66(3): 776–789. https://doi.org/10.1016/j.ympev.2012.11.002

Shen X, Tian M, Yan B, Chu K (2015) Phylomitogenomics of Malacostraca (Arthropoda: Crustacea). Acta Oceanologica Sinica 34(2): 84–92. https://doi.org/10.1007/s13131-015-0583-1

Shi GH, Cui ZX, Hui M, Liu Y, Chan TY, Song CW (2015) The complete mitochondrial genomes of *Umalia orientalis* and *Lyreidus brevifrons*: The phylogenetic position of the family Raninidae within brachyuran crabs. Marine Genomics 21: 53–61. https://doi.org/10.1016/j.margen.2015.02.002

Shi GH, Cui ZX, Hui M, Liu Y, Chan TY, Song CW (2016) Unusual sequence features and gene rearrangements of primitive crabs revealed by three complete mitochondrial genomes of Dromiacea. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 20: 65–73. https://doi.org/10.1016/j.cbd.2016.07.004

Shih HT, Huang C, Ng PKL (2016) A re-appraisal of the widely-distributed freshwater crab genus *Sinopotamon* Bott, 1967, from China, with establishment of a new genus (Crustacea: Decapoda: Potamidae). Zootaxa 4138: 309–331. https://doi.org/10.11646/zootaxa.4138.2.5

Song SN, Tang P, Wei SJ, Chen XX (2016) Comparative and phylogenetic analysis of the mitochondrial genomes in basal hymenopterans. Scientific Reports 6: 20972. https://doi.org/10.1038/srep20972

Stamatakis A (2006a) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis A (2006b) Phylogenetic models of rate heterogeneity: a high performance computing perspective. Parallel and Distributed Processing Symposium, 2006. IPDPS 2006. 20th International IEEE 2006: 8. https://doi.org/10.1109/IPDPS.2006.1639535

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sun HY, Zhou KY, Song DX (2003) Mitochondrial genome and phylogenetic reconstruction of arthropods. Zoological Research 24(6): 467–479.

Sun HY, Zhou KY, Song DX (2005) Mitochondrial genome of the Chinese mitten crab *Eriocheir japonica sinenesis* (Brachyura: Thoracotremata: Grapsoidea) reveals a novel gene order and two target regions of gene rearrangements. Gene 349: 207–217. https://doi.org/10.1016/j.gene.2004.12.036

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30(12): 2725–2729 https://doi.org/10.1093/molbev/mst197

Tan MH, Gan HM, Lee YP, Austin CM (2016a) The complete mitogenome of the swimming crab *Thalamita crenata* (Rüppell, 1830) (Crustacea; Decapoda; Portunidae). Mitochondrial DNA Part A 27(2): 1275–1276. http://dx.doi.org/10.3109/19401736.2014.945553

Tan MH, Gan HM, Lee YP, Austin CM (2016b) The complete mitogenome of the moon crab *Ashtoret lunaris* (Forskal, 1775), (Crustacea; Decapoda; Matutidae). Mitochondrial DNA Part A 27(2): 1313–1314. http://dx.doi.org/10.3109/19401736.2014.945572

Tan MH, Gan HM, Lee YP, Austin CM (2016c) The complete mitogenome of the stone crab *Myomenippe fornasinii* (Bianconi, 1851) (Crustacea: Decapoda: Menippidae). Mitochondrial DNA Part A 27(2): 1374–1375. http://dx.doi.org/10.3109/19401736.2014.947587

Tan MH, Gan HM, Lee YP, Austin CM (2016d) The complete mitogenome of the soldier crab *Mictyris longicarpus* (Latreille, 1806)(Crustacea: Decapoda: Mictyridae). Mitochondrial DNA Part A 27(3): 2121–2122. http://dx.doi.org/10.3109/19401736.2014.982585

Tan MH, Gan HM, Lee YP, Austin CM (2016e) The complete mitogenome of the ghost crab *Ocypode ceratophthalmus* (Pallas, 1772)(Crustacea: Decapoda: Ocypodidae). Mitochondrial DNA Part A 27(3): 2123–2124. http://dx.doi.org/10.3109/19401736.2014.982587

Tan MH, Gan HM, Lee YP, Austin CM (2016f) The complete mitogenome of purple mottled shore crab *Cyclograpsus granulosus* H. Milne-Edwards, 1853 (Crustacea: Decapoda: Grapsoidea). Mitochondrial DNA Part A 27(6): 3981–3982. https://doi.org/10.3109/19401736.2014.989514

Tan MH, Gan HM, Schultz MB, Austin CM (2015) MitoPhAST, a new automated mitogenomic phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes

including eight new freshwater crayfish mitogenomes. Molecular Phylogenetics and Evolution 85: 180–188. https://doi.org/10.1016/j.ympev.2015.02.009

Tan MH, Gan HM, Lee YP, Poore GC, Austin CM (2017) Digging deeper: new gene order rearrangements and distinct patterns of codons usage in mitochondrial genomes among shrimps from the Axiidea, Gebiidea and Caridea (Crustacea: Decapoda). Peerj 5: e2982. https://doi.org/10.7717/peerj.2982

Tsang LM, Schubart CD, Ahyong ST, Lai JC, Au EY, Chan TY, Ng PK, Chu KH (2014) Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. Molecular Biology and Evolution 31: 1173–1187. https://doi.org/10.1093/molbev/msu068

van Bakel BWM, Guinot D, Artal P, Fraaije RHB, Jagt JWM (2012) A revision of the Palaeocorystoidea and the phylogeny of raninoidian crabs (Crustacea, Decapoda, Brachyura, Podotremata). Zootaxa 3215: 1–216. http://www.mapress.com/zootaxa/2012/f/z03215p216f.pdf

Von Sternberg R, Cumberlidge N, Rodriguez G (1999) On the marine sister groups of the freshwater crabs (Crustacea: Decapoda). Journal of Zoological Systematics and Evolutionary Research 37(1): 19–38. https://doi.org/10.1046/j.1439-0469.1999.95092.x

Von Sternberg R, Cumberlidge N (2001) On the Heterotreme-Thoracotreme distinction in the Eubrachyura de Saint Laurent, 1980 (Decapoda, Brachyura). Crustaceana 74: 321–338. https://doi.org/10.1163/156854001300104417

Wang Y, Zeng QW, Zou JX, Zhu CC, Bai J, Shih TH, Cheng SL, Zhou XM (2016) The complete mitochondrial genome of freshwater crab *Sinopotamon xiushuiense* (Decapoda: Brachyura: Potamoidea). Mitochondrial DNA Part B 1: 750–752. http://dx.doi.org/10.1080/23802359.2016.1209094

Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20: 3252–3255. doi: https://doi.org/10.1093/bioinformatics/bth352

Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. Journal of Heredity 92: 371–373. https://doi.org/10.1093/jhered/92.4.371

Xing YH, Ma XP, Wei YQ, Pan D, Liu WL, Sun HY (2016) The complete mitochondrial genome of the semiterrestrial crab, *Chiromantes neglectum* (Eubrachyura: Grapsoidea: Sesarmidae). Mitochondrial DNA Part B 1: 461–463. https://doi.org/10.1080/23802359.2016.1186509

Yamauchi MM, Miya MU, Nishida M (2003) Complete mitochondrial DNA sequence of the swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura). Gene 311: 129–135. https://doi.org/10.1016/S0378-1119(03)00582-1

Yang JS, Lu B, Chen DF, Yu YQ, Yang F, Nagasawa H, Yang WJ (2013) When did decapods invade hydrothermal vents? Clues from the Western Pacific and Indian Oceans. Molecular Biology and Evolution 30(2): 305–309. https://doi.org/10.1093/molbev/mss224

Yang JS, Nagasawa H, Fujiwara Y, Tsuchida S, Yang WJ (2010) The complete mitogenome of the hydrothermal vent crab *Gandalfus yunohana* (Crustacea: Decapoda: Brachyura): a link between the Bythograeoidea and Xanthoidea. Zoologica Scripta 39(6): 621–630. https://doi.org/10.1111/j.1463-6409.2010.00442.x

Yang JS, Yang WJ (2008) The complete mitochondrial genome sequence of the hydrothermal vent galatheid crab *Shinkaia crosnieri* (Crustacea: Decapoda: Anomura): a novel arrangement and incomplete tRNA suite. BMC Genomics 9(1): 257. https://doi.org/10.1186/1471-2164-9-257

Yeo DC, Ng PKL, Cumberlidge N, Magalhães C, Daniels SR, Campos MR (2008) Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. Hydrobiologia 595: 275–286. https://doi.org/10.1007/s10750-007-9023-3

Yu YQ, Ma WM, Yan WJ Yang JS (2014) The complete mitogenome of the lined shore crab *Pachygrapsus crassipes* Randall 1840 (Crustacea: Decapoda: Grapsidae). Mitochondrial DNA 25(4): 263–264. https://doi.org/10.3109/19401736.2013.800497

Supplementary material 1

Figure S1
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Data type: TIF image file
Explanation note: Comparisons of the consensus sequence and variable sites in the entire mNCR for *Sinopotamon yaanense*, *S. yangtsekiense* and *S. xiushuiense*. The conserved central domain is grey shaded, and the extended termination associated sequences (ETAS) is underlined.

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